

REMARKS

Claims 46-73 have been canceled without prejudice or disclaimer. Claims 74-106 have been added and therefore are pending. Claims 74-106 are based on claims 46-73.

Applicants enclose a paper copy and a computer readable form of a Sequence Listing. The content of the paper copy and of the computer readable form is the same. The specification has been amended to provide SEQ ID NOS for the sequences disclosed therein. This submission contains no new matter.

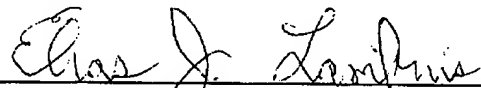
The specification also has been amended to correct obvious errors. As described in Example 2, the mannanase produced in that example has an N-terminal amino acid at position 32 of SEQ ID NO: 2. Also, the specification specifies that the catalytic domain of the mannanase of SEQ ID NO: 2 ends at position 330. For the above reasons, Applicants submit that the correction of the N-terminal amino acid and the amino acid at the end of the catalytic domain does not constitute new matter.

It is respectfully submitted that the present amendment presents no new issues or new matter and places this case in condition for allowance. Reconsideration of the application in view of the above amendments and the following remarks is requested.

The Examiner is hereby invited to contact the undersigned by telephone if there are any questions concerning this amendment or application.

Respectfully submitted,

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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Serial No.: 09/339,159

Group Art Unit: 1652

Filed: June 24, 1999

Examiner: M. Rao

For: Mannanases

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Sir:

Below is a marked-up version of the amendments made in the accompanying amendment.

IN THE SPECIFICATION:

The paragraph on page 3, line 24 – page 4, line 1 has been amended as follows:

In a first aspect, the present invention relates to a mannanase which is i) a polypeptide produced by *Bacillus* sp. 1633, ii) a polypeptide comprising an amino acid sequence as shown in positions ~~31-330~~ 32-330 of SEQ ID NO:2, or iii) an analogue of the polypeptide defined in i) or ii) which is at least 65% homologous with said polypeptide, is derived from said polypeptide by substitution, deletion or addition of one or several amino acids, or is immunologically reactive with a polyclonal antibody raised against said polypeptide in purified form.

The paragraph on page 4, lines 2-12 has been amended as follows:

Within one aspect, the present invention provides an isolated polynucleotide molecule selected from the group consisting of (a) polynucleotide molecules encoding a polypeptide having mannanase activity and comprising a sequence of nucleotides as shown in SEQ ID NO: 1 from nucleotide ~~91-94~~ 94 to nucleotide 990; (b) species homologs of (a); (c) polynucleotide molecules that encode a polypeptide having mannanase activity that is at least 65% identical to the amino acid sequence of SEQ ID NO: 2 from amino acid residue ~~31-32~~ 32 to amino acid residue 330; (d) molecules complementary to (a), (b) or (c); and (e) degenerate nucleotide sequences of (a), (b), (c) or (d).

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The paragraph on page 4, line 22 – page 5, line 2 has been amended as follows:

Within another aspect of the invention there is provided an expression vector comprising the following operably linked elements: a transcription promoter; a DNA segment selected from the group consisting of (a) polynucleotide molecules encoding a polypeptide having mannanase activity and comprising a sequence of nucleotides as shown in SEQ ID NO: 1 from nucleotide 94 to nucleotide 990; (b) species homologs of (a); (c) polynucleotide molecules that encode a polypeptide having mannanase activity that is at least 65% identical to the amino acid sequence of SEQ ID NO: 2 from amino acid residue 31-32 to amino acid residue 330; and (d) degenerate nucleotide sequences of (a), (b), or (c); and a transcription terminator.

The paragraph on page 5, lines 7-17 has been amended as follows:

Further aspects of the present invention provide an isolated polypeptide having mannanase activity selected from the group consisting of (a) polypeptide molecules comprising a sequence of amino acid residues as shown in SEQ ID NO:2 from amino acid residue 31-32 to amino acid residue 330; (b) species homologs of (a); and a fusion protein having mannanase activity comprising a first polypeptide part exhibiting mannanase activity and a second polypeptide part exhibiting cellulose binding function, the second polypeptide preferably being a cellulose binding domain (CBD), such as a fusion protein represented by SEQ ID NO:4.

The paragraph on page 24, line 27 – page 25, line 12 has been amended as follows:

The sequence of amino acids in positions 31-490-32-490 of SEQ ID NO: 2 is a mature mannanase sequence. The sequence of amino acids nos. 4-30-1-31 of SEQ ID NO: 2 is the signal peptide. It is believed that the subsequence of amino acids in positions 31-330-32-330 of SEQ ID NO: 2 is the catalytic domain of the mannanase enzyme and that the mature enzyme additionally comprises a linker in positions 331-342 and at least one C-terminal domain of unknown function in positions 343-490. Since the object of the present invention is to obtain a polypeptide which exhibits mannanase activity, the present invention relates to any mannanase enzyme comprising the sequence of amino acids nos. 31-330-32-330 of SEQ ID NO: 2, ie a catalytical domain, optionally operably linked, either N-terminally or C-terminally, to one or two or

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more than two other domains of a different functionality. The domain having the subsequence of amino acids nos. 343-490 of SEQ ID NO: 2 is a domain of the mannanase enzyme of unknown function, this domain being highly homologous with similar domains in known mannanases, cf. example 1.

The paragraph on page 29, line 26 – page 30, line 28 has been amended as follows:

The present invention also provides mannanase polypeptides that are substantially homologous to the polypeptides of SEQ ID NO:2, SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30 and SEQ ID NO:32, respectively, and species homologs (paralogs or orthologs) thereof. The term "substantially homologous" is used herein to denote polypeptides having 65%, preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, and even more preferably at least 90%, sequence identity to the sequence shown in amino acids nos. ~~33-340~~32-330 or nos. ~~33-490~~32-490 of SEQ ID NO:2 or their orthologs or paralogs; or to the sequence shown in amino acids nos. 32-344 or nos. 32-494 of SEQ ID NO:6 or their orthologs or paralogs; or to the sequence shown in amino acids nos. 32-362 or nos. 32-586 of SEQ ID NO:10 or their orthologs or paralogs; or to the sequence shown in amino acids nos. 33-331 of SEQ ID NO:12 or its orthologs or paralogs; or to the sequence shown in amino acids nos. 166-488 or nos. 22-488 of SEQ ID NO:14 or their orthologs or paralogs; or to the sequence shown in amino acids nos. 68-369 or nos. 32-369 of SEQ ID NO:16 or their orthologs or paralogs; or to the sequence shown in amino acids nos. 1-305 of SEQ ID NO:18 or its orthologs or paralogs; or to the sequence shown in amino acids nos. 1-132 of SEQ ID NO:20 or its orthologs or paralogs; or to the sequence shown in amino acids nos. 29-320 of SEQ ID NO:22 or its orthologs or paralogs; or to the sequence shown in amino acids nos. 29-188 of SEQ ID NO:24 or its orthologs or paralogs; or to the sequence shown in amino acids nos. 301-625 or nos. 30-625 of SEQ ID NO:26 or their orthologs or paralogs; or to the sequence shown in amino acids nos. 166-496 or nos. 38-496 of SEQ ID NO:28 or their orthologs or paralogs; or to the sequence shown in amino acids nos. 26-361 of SEQ ID NO:30 or its orthologs or paralogs; or to the sequence shown in amino acids nos. 593-903 or nos. 23-903 of SEQ ID NO:32 or their orthologs or paralogs.

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The paragraph on page 30, line 29 – page 31, line 22 has been amended as follows:

Such polypeptides will more preferably be at least 95% identical, and most preferably 98% or more identical to the sequence shown in amino acids nos. ~~31-330~~ 32-330 or nos. ~~31-490~~ 32-490 of SEQ ID NO:2 or its orthologs or paralogs; or to the sequence shown in amino acids nos. 32-344 or nos. 32-494 of SEQ ID NO:6 or its orthologs or paralogs; or to the sequence shown in amino acids nos. 32-362 or nos. 32-586 of SEQ ID NO:10 or its orthologs or paralogs; or to the sequence shown in amino acids nos. 33-331 of SEQ ID NO:12 or its orthologs or paralogs; or to the sequence shown in amino acids nos. 166-488 or nos. 22-488 of SEQ ID NO:14 or its orthologs or paralogs; or to the sequence shown in amino acids nos. 68-369 or nos. 32-369 of SEQ ID NO:16 or its orthologs or paralogs; or to the sequence shown in amino acids nos. 1-305 of SEQ ID NO:18 or its orthologs or paralogs; or to the sequence shown in amino acids nos. 1-132 of SEQ ID NO:20 or its orthologs or paralogs; or to the sequence shown in amino acids nos. 29-320 of SEQ ID NO:22 or its orthologs or paralogs; or to the sequence shown in amino acids nos. 29-188 of SEQ ID NO:24 or its orthologs or paralogs; or to the sequence shown in amino acids nos. 301-625 or nos. 30-625 of SEQ ID NO:26 or its orthologs or paralogs; or to the sequence shown in amino acids nos. 166-496 or nos. 38-496 of SEQ ID NO:28 or its orthologs or paralogs; or to the sequence shown in amino acids nos. 26-361 of SEQ ID NO:30 or its orthologs or paralogs; or to the sequence shown in amino acids nos. 593-903 or nos. 23-903 of SEQ ID NO:32 or its orthologs or paralogs.

The paragraph on page 38, lines 8-21 has been amended as follows:

Using the methods discussed above, one of ordinary skill in the art can identify and/or prepare a variety of polypeptides that are substantially homologous to residues ~~33-340~~ 32-330 or ~~33-490~~ 32-490 of SEQ ID NO:2; or to residues 32-344 or 32-494 of SEQ ID NO:6; or to residues 32-362 or 32-586 of SEQ ID NO:10; or to residues 33-331 of SEQ ID NO:12; or to residues 166-488 or 22-488 of SEQ ID NO:14; or to residues 68-369 or 32-369 of SEQ ID NO:16; or to residues 1-305 of SEQ ID NO:18; or to residues 1-132 of SEQ ID NO:20; or to residues 29-320 of SEQ ID NO:22; or to residues 29-188 of SEQ ID NO:24; or to residues 301-625 or 30-625 of SEQ ID NO:26; or to residues 166-496 or 38-496 of SEQ ID NO:28; or to residues 26-361 of SEQ ID NO:30; or to residues 593-903 or 23-903 of SEQ ID NO:32 and retain the mannanase activity of the wild-type protein.

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The paragraph on page 91, lines 22-25 has been amended as follows:

The two PCR primers used have the following sequences:

#LWN5494 5'-GTCGCCGGGGCGGCCGCTATCAATTGGTAACTGTATCTCAGC -3' (SEQ ID NO: 35)
#LWN5495 5'-GTCGCCGGGGAGCTCTGATCAGGTACCAAGCTTGTCGACCTGCAGAA
TGAGGCAGCAAGAAGAT -3' (SEQ ID NO: 36)

The paragraph on page 92, lines 1-16 has been amended as follows:

This cloning replaces the first amyL promoter cloning with the same promoter but in the opposite direction. The two primers used for PCR amplification have the following sequences:

#LWN5938 5'-GTCGGCGGCCGCTGATCACGTACCAAGCTTGTCGACCTGCAGAATG
AGGCAGCAAGAAGAT -3' (SEQ ID NO: 37)
#LWN5939 5'-GTCGGAGCTCTATCAATTGGTAACTGTATCTCAGC -3' (SEQ ID NO: 38)

The plasmid pSJ2670 was digested with the restriction enzymes PstI and BclI and a PCR fragment amplified from a cloned DNA sequence encoding the alkaline amylase SP722 (Patent # WO 9526397-A1) was digested with PstI and BclI and inserted to give the plasmid pMOL944. The two primers used for PCR amplification have the following sequence:

#LWN7864 5' -AACAGCTGATCACGACTGATCTTTTAGCTTGGCAC-3' (SEQ ID NO: 39)
#LWN7901 5' -AACTGCAGCCGCGGCACATCATAATGGGACAAATGGG -3' (SEQ ID NO: 40)

The paragraph on page 97, lines 13-21 has been amended as follows:

The mannanase encoding DNA sequence of the invention was PCR amplified using the PCR primer set consisting of the following two oligo nucleotides:

BXM2.upper.SacII

5'-GTT GAG AAA GCG GCC GCC TTT TTT CTA TTC TAC AAT CAC ATT ATC-3' (SEQ ID NO: 41)

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BXM2.core.lower.NotI

5'-GAC GAC GTA CAA GCG GCC GCT CAC TAC GGA GAA GTT CCT CCA TCA G-3' (SEQ ID NO: 42)

The paragraph on page 98, line 29 – page 99, line 9 has been amended as follows:

One such positive clone was restreaked several times on agar plates as used above, this clone was called MB748. The clone MB748 was grown overnight in TY-10 µg/ml Kanamycin at 37°C, and next day 1 ml of cells were used to isolate plasmid from the cells using the Qiaprep Spin Plasmid Miniprep Kit #27106 according to the manufacturers recommendations for B. subtilis plasmid preparations. This DNA was DNA sequenced and revealed the DNA sequence corresponding to the mature part of the mannanase (corresponding to positions 91-990-94-990 of ~~in the appended DNA sequence SEQ ID NO:1~~ and positions 31-330-32-330 ~~in the appended protein sequence of SEQ ID NO:2~~) with introduced stop codon replacing the amino acid residue no 331 corresponding to the base pair positions 1201-1203 in SEQ ID NO:1.

The paragraph on page 99, lines 13-21 has been amended as follows:

The mannanase encoding DNA sequence of the invention was PCR amplified using the PCR primer set consisting of these two oligo nucleotides:

BXM2.upper.SacII

5'-CAT TCT GCA GCC GCG GCA AAT TCC GGA TTT TAT GTA AGC GG-3' (SEQ ID NO: 43)

BXM2.lower.NotI

5'-GTT GAG AAA GCG GCC GCC TTT TTT CTA TTC TAC AAT CAC ATT ATC -3' (SEQ ID NO: 44)

The paragraph on page 103, lines 8-9 has been amended as follows:

The following N-terminal sequence of the purified protein was determined: ANSGFYVSGTTLYDANG (amino acids 32-48 of SEQ ID NO: 2).

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The paragraph on page 104, line 28 – page 105, line 8 has been amended as follows:

The pMB993 vector contains the CipB CBD with a peptide linker preceeding the CBD. The linker consists of the following peptide sequence ASPEPTPEPT (SEQ ID NO: 49) and is directly followed by the CipB CBD. The AS amino acids are derived from the DNA sequence that constitutes the Restriction Endonuclease site NheI, which in the following is used to clone the mannase of the invention.

Mannanase.Upper.SacII

5'-CAT TCT GCA GCC GCG GCA AAT TCC GGA TTT TAT GTA AGC GG -3' (SEQ ID NO: 45)

Mannanase.Lower.NheI

5'-CAT CAT GCT AGC TGT AAA AAC GGT GCT TAA TCT CG -3' (SEQ ID NO: 46)

The paragraph on page 109, lines 1-6 has been amended as follows:

Mannanase.upper.SacII

5'-CAT TCT GCA GCC GCG GCA GCA AGT ACA GGC TTT TAT GTT GAT GG-3' (SEQ ID NO: 47)

Mannanase.lower.NotI

5'-GAC GAC GTA CAA GCG GCC GCG CTA TTT CCC TAA CAT GAT GAT ATT TTC G -3' (SEQ ID NO: 48)

The paragraph on page 116, lines 21-24 has been amended as follows:

BXM1.upper.SacII

5'- CAT TCT GCA GCC GCG GCA TTT TCT GGA AGC GTT TCA GC-3' (SEQ ID NO: 50)

BXM1.lower.NotI

5'-CAG CAG TAG CGG CCG CCA CTT CCT GCT GGT ACA TAT GC -3' (SEQ ID NO: 51)

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The paragraph on page 122, lines 23-28 has been amended as follows:

BXM5.upper.SacII

5'-CAT TCT GCA GCC GCG GCA CAT CAC AGT GGG TTC CAT G-3' (SEQ ID NO: 52)

BXM5.lower.NotI

5'-GCG TTG AGA CGC GCG GCC GCT TAT TGA AAC ACA CTG CTT CTT TTA G-3' (SEQ ID NO: 53)

The paragraph on page 126, lines 16-21 has been amended as follows:

BXM7.upper.SacII

5'-CAT TCT GCA GCC GCG GCA AGT GGA CAT GGG CAA ATG C-3' (SEQ ID NO: 54)

BXM7.lower.NotI

5'-GCG TTG AGA CGC GCG GCC GCT TAT TTT TTG TAT ACA CTA ACG ATT TC-3' (SEQ ID NO: 55)

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